

Amendments to the Specification:

Page 1, replace the heading in line 1 with the following amended heading:

SPECIFICATION    DESCRIPTION.

Please replace the paragraph bridging pages 1 and 2 with the following amended paragraph:

Sulfur is one of elements which take important roles in nature. Particularly, it takes a substantial role as a constituting element for cysteine and methionine as sulfur-containing amino acids. Further, it has been known that a large sulfur cycle is carried out between plants and animals. In the plants, sulfur is taken in the form of sulfate ions and reduced to sulfide ions, and then subjected to cysteine synthesis and further methionine synthesis. In the animals, methionine is ingested from foods by food ~~eyele~~ chain and metabolized to cysteine in vivo. In this metabolization stage, homocysteine is formed as an intermediate.

Page 2, please replace the paragraph beginning on line 7 with the following amended paragraph:

Homocysteine is an intermediary metabolite which is scarcely present at the normal condition. However, it has been reported that when its concentration in blood is at a high level, the rate of occurrence of coronary disease and cerebral apoplexy is high. Accordingly, the homocysteine amount in blood ~~has been~~ is already recognized or still unrecognized to be a risk factor useful for predicting the occurrence of thromboembolism such as ~~myocardial~~ myocardial infarction or cerebral infarction, or arteriosclerosis.

Please replace the paragraph bridging pages 2 and 3 with the following amended paragraph:

Furthermore, ~~an enzyme is~~ enzymes are known which ~~has~~ have a function of acting on homocysteine or cysteine and conducting decomposition or substitution, thereby forming hydrogen sulfide. However, even if it is attempted to quantitatively determine homocysteine or cysteine by utilizing such ~~an enzyme~~ enzymes, since there is no method for measuring the formed hydrogen sulfide conveniently with high sensitivity, no practical method

has been found in which ~~this enzyme is~~ these enzymes are used for quantitatively determining homocysteine or cysteine.

Please replace the paragraph bridging pages 4 and 5 with the following amended paragraph:

The present invention further provides a method for quantitatively determining a specific substance, which comprises adding to a sample containing [[a]] the specific substance, a component which acts on the specific substance so that the specific substance forms hydrogen sulfide or sulfide ions, metal ions or a compound which liberates said metal ions, and a metal indicator which reacts with the metal ions and resultingly undergoes color development, wherein the color development is accelerated or inhibited by the hydrogen sulfide or sulfide ions; and measuring the degree of color development of the metal indicator.

Please replace the paragraph bridging pages 6 and 7 with the following amended paragraph:

In the method for quantitatively determining hydrogen sulfide or sulfide ions of the present invention, the case of

utilizing the action of hydrogen sulfide or sulfide ions which ~~inhibits~~ inhibit the color development reaction between ~~[[the]]~~ metal ions and ~~[[the]]~~ a metal indicator, will be described hereinbelow. That is, the hydrogen sulfide or sulfide ions present in a sample are allowed to contact with metal ions to form a metal sulfide, and at the same time, the metal ions are reacted with the metal indicator, and then the degree of color development is measured, whereby the amount of the complexes formed by the reaction between the metal ions and the metal indicator is determined. Then, the above amount of the formed complexes is subtracted from the amount of the formed complexes when the metal ions are reacted with the metal indicator under the same conditions as in the above, provided that no hydrogen sulfide or sulfide ions are present, whereby the decreased amount of the formed complexes is obtained by utilizing the characteristic that the metal sulfide forms no complexes. The decreased amount of the formed complexes corresponds to the amount of the hydrogen sulfide or sulfide ions present in the sample. Accordingly, the amount of the hydrogen sulfide or sulfide ions in the sample can be calculated based on the decreased amount of the formed complexes.

Please replace the paragraph bridging pages 7 and 8 with the following amended paragraph:

The case of utilizing the action of hydrogen sulfide or sulfide ions which accelerates the color development reaction between the metal ions and the metal indicator, will be described hereinbelow. That is, into the sample containing the hydrogen sulfide or sulfide ions, the metal ions and the metal indicator are added, and a reaction is carried out, and then the degree of color development is measured to determine the amount of the ~~formed~~ complexes formed by the reaction between the metal ions and the metal indicator. Then, from this amount of the formed complexes, the amount of the ~~formed~~ complexes formed when the metal ions are reacted with the metal indicator under the same conditions as in the above, provided that no hydrogen sulfide or sulfide ions are present, is subtracted, whereby the increased amount of the formed complexes is determined. Since the increased amount of the formed complexes is attributable to the acceleration of the reaction between the metal ions and the metal indicator by the hydrogen sulfide or sulfide ions, it corresponds to the amount of the hydrogen sulfide or sulfide ions. Accordingly, the amount of the hydrogen sulfide or sulfide ions in the sample can be calculated based on the increased amount of the formed complexes.

Please replace the paragraph bridging pages 8 and 9 with the following amended paragraph:

As the metal indicator used in the present invention, there is no particular limitation so long as it is a substance of which the color development reaction with the metal ions is inhibited or accelerated by the hydrogen sulfide or sulfide ions. Preferred is the one having a high sensitivity of color development at the time of forming the complexes. For example, pyridylazo compounds and nitrosoaminophenol compounds are preferably used. Further specifically, as the pyridylazo compounds, 2-(5-bromo-2-pyridylazo)-5-[N-[[N]]n-propyl-N-(3-sulfopropyl)amino]phenol sodium salt (trade name: 5Br·PAPS; hereinafter simply referred to as 5Br·PAPS) and 2-(5-nitro-2-pyridylazo)-5-[N-[[N]]n-propyl-N-(3-sulfopropyl)-amino]phenol sodium salt (trade name: Nitro·PAPS) are preferably used. Further, as the nitrosoaminophenol compounds, 2-nitroso-5-[N-N-propyl-N-(3-sulfopropyl)-amino]phenol (trade name: Nitroso·PSAP) and 2-nitroso-5-[N-ethyl-N-(3-sulfopropyl)amino]phenol (trade name: Nitroso·ESAP) are preferably used. These compounds are water soluble and have a property that they form complexes with zinc ions, copper ions, cobalt ions or iron ions and undergo color development with a

high sensitivity. As these metal indicators, ones having various characteristics are commercially sold and available from, for example, ~~Kabushiki Kaisha Dojin Kagaku Kenkyusho~~ DOJINDO LABORATORIES.

Page 10, please replace the paragraph beginning on line 6 with the following amended paragraph:

L-methionine- $\gamma$ -lyase is known as an enzyme that shows a decomposition (liberation) action against homocysteine and forms hydrogen sulfide in the absence of a thiol compound, but shows an action of catalyzing  $\gamma$ -substitution in the presence of a thiol compound. This enzyme is obtainable from microorganisms which are capable of producing it, for example, bacteria of the Pseudomonas genus, but some types thereof ~~may be~~ are commercially sold and available from, for example, Wako ~~Junyaku Kabushiki Kaisha~~ Pure Chemical Industries, Ltd..

Page 10, please replace the paragraph beginning on line 15 with the following amended paragraph:

Further, O-acetylhomoserine-lyase is known as an enzyme that has an amino acid-synthesizing action (for example, an action of forming homocysteine from O-acetylhomoserine and hydrogen

sulfide, and methionine from methanethiol) (~~reference can be made to see the~~ "Handbook of Enzyme", compiled by Maruo et al, Asakura Shoten, 1982). The present inventors have newly found an enzymatic action that when *O*-acetylhomoserine-lyase is allowed to act on homocysteine in the presence of a thiol compound, hydrogen sulfide is formed by  $\gamma$ -substitution (Japanese Patent Application No. 10-347003).

Please replace the paragraph bridging pages 10 and 11 with the following amended paragraph:

With respect to *O*-acetylhomoserine-lyase, various microorganisms that are capable of producing it have been known (~~reference can be made to see~~, for example, Ozaki et al, J. Biochem 91; 1163-1171 (1982), Yamagata, J. Biochem 96; 1511-1523 (1984), Brzywczy et al, Acta. Biochimica. Polonica 40(3); 421-428 (1993)). *O*-Acetylhomoserine-lyase may be obtained by culturing these microorganisms, but some types thereof ~~may be~~ are commercially sold and available from, for example, Yunitica Ltd. These enzymes (E1) have a function of strongly acting on homocysteine and acting a little on cysteine, thereby forming hydrogen sulfide.



Page 12, please replace the paragraph beginning on line 4 with the following amended paragraph:

Further, when the specific substance is cysteine, as the component for forming hydrogen sulfide or sulfide ions from the specific substance, an enzyme (E2) which has a function of acting on cysteine so that the cysteine forms hydrogen sulfide or sulfide ions, is used. As such an enzyme, for example, *O*-acetylserine-lyase,  $\beta$ -cyanoalanine synthase and cysteine lyase may be mentioned. Particularly, *O*-acetylserine-lyase ~~may~~ is preferably ~~be~~ used.

Page 13, please replace the first paragraph with the following amended paragraph:

For example, the following are physicochemical properties of *O*-acetylserine-lyase obtained from spinach by the method described in Yamaguchi et al, ~~Biochem~~ Biochim. Biophys. Acta 1251; 91-98(1995). Among the following physicochemical properties, items other than molecular weight were determined by the present inventors.

Please replace the paragraph bridging pages 19 and 20 with the following amended paragraph:

Sample: an aqueous solution containing from 0 to 100  $\mu\text{M}$  of sodium sulfide (manufactured by Wako ~~Junyaku~~ ~~[[K.K.]]~~ Pure Chemicals Industries, Ltd.) as sulfide ions.

1st reagent:

Tris buffer (pH 8.5) 100 mM

Zinc chloride 10  $\mu\text{M}$

2nd reagent:

5Br-PAPS (manufactured by ~~Dojin Kagaku Kenkyusho~~ ~~[[K.K.]]~~ DOJINDO LABORATORIES) 1 mM